Attorney Docket Number OC01600

PATENT APPLICATION

METHODS FOR TREATING DISORDERS OF CALCIUM HOMEOSTASIS

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File No. OC01600-US

METHODS FOR TREATING DISORDERS OF CALCIUM HOMEOSTASIS

This application claims the benefit of U.S. Provisional Patent Application No. 60/414,948, filed September 30, 2002, which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to methods for treating calcium homeostatic disorders in a subject.

BACKGROUND OF THE INVENTION

The complex interplay between calcitropic hormones and their Ca^{2+}-translocating target tissues is crucial for maintaining the extracellular/serum calcium concentration in humans within its normal range. These hormones include, *inter alia*, the parathyroid hormone (PTH); others include calcitonin and 1,25-dihydroxyvitamin D_3. The actions of these hormones regulate renal Ca^{2+} reabsorption, intestinal Ca^{2+} absorption and skeletal Ca^{2+} mobilization. A key element in this process is a G-protein coupled receptor (GPCR) termed the Calcium Ion Sensing Receptor (CaSR). Human CaSR was originally cloned from the parathyroid gland and was subsequently identified in several other tissues including the kidney (Aida, *et al.*, (1995) Biochem. Biophys. Res. Comm. 214:524-529 and Garrett, *et al.*, (1995) J. Biol. Chem. 270:12919-12925). Furthermore, CaSR was identified in several species including *X.laevis*, rabbits, rats and cows (Brown, *et al.*, (1993) Nature 366:575-580; Riccardi, *et al.*, (1995) Proc. Natl. Acad. Sci. 92:131-135 and Butters, *et al.*, (1997) J. Bone Mineral Res. 12:568-579). CaSR may regulate serum calcium levels by modulating the level of synthesis and secretion of PTH and by modulating the extent of Ca^{2+} reabsorption in the kidneys (Chattopadhyay, (2000) Int. J. Biochem. Cell Bio. 32:789-804; Chattopadhyay, *et al.*, (2000) Cellular Signal. 12:361-366).

An example of a calcium homeostatic disorder is familial hypocalciuric hypercalcemia (FHH), which is sometimes called familial benign hypercalcemia (FBH) or familial benign hypocalciuric hypercalcemia (FBHH). Subjects with FHH suffer from lifelong mild to moderate hypercalcemia. As those with FHH age, they can develop calcium deposits in their cartilage and suffer bouts of acute pancreatitis (Van haeften, *et*

al., (1994) Neth. J. Med. 45(3):110-113; Davies, *et al.*, (1981) Br. Med. J. (Clin. Res. Ed.) 282(6269):1023-1025).

Neonatal severe hyperparathyroidism (NSHPT) is a condition which can lead to marked bony demineralization, multiple fractures and rib cage malformation. This condition can be fatal if a parathyroidectomy is not carried out within the first few weeks of life (Chattopadhyay, *et al.*, (1996) Endocrin. Rev. 17(4): 289-307). NSHPT may be linked to decreased CaSR activity which, in some cases, may be associated with a CaSR mutation (Chattopadhyay, *et al.*, (1996) Endocrine Rev. 17: 289-307; Heath, *et al.*, (1996) J. Clin. Endocrin. Metab. 81:1312-1317; Pearce, *et al.*, (1995) J. Clin. Invest. 96:2683-2692 and Pollak, *et al.*, (1993) Cell 75: 1297-1303).

Renal secondary hyperparathyroidism is characterized by an excess of serum PTH which is a secondary effect caused by renal failure. Subjects with renal secondary hyperparathyroidism can suffer from disabling skeletal diseases such as renal osteodystrophy (Sherrard, *et al.*, (1993) Kidney Int. 43:436-442; Torres, *et al.*, (1995) Kidney Int. 47: 1434-1442; Moniere-Faugere, *et al.*, (1996) Nephrol. Dial. Transplant. 11: 111-120).

One of the most common causes of hypercalcemia is malignancy (*i.e.*, malignancy associated hypercalcemia (MAH) or humoral hypercalcemia of malignancy (HHM)). It is estimated that 10%-20% of cancer patients suffer from hypercalcemia. The most common cancers that are associated with the development of MAH are squamous cell lung cancer, squamous cell head and neck cancers, breast cancer, multiple myeloma, T-cell lymphomas, renal cell cancer and ovarian cancer. A proximal cause of MAH is believed to be increased bone resorption. Subjects suffering from MAH can suffer from nausea, vomiting, lethargy, confusion and, eventually, death.

Parathyroid hormone has been known since the 1930s to have catabolic effects in bone (*e.g.*, causing resorption of calcium from bone to serum). One medical condition which is characterized by increased bone resorption and increased frequency of bone fractures is osteoporosis. Excess serum parathyroid hormone is likely to be an exacerbating factor in osteoporosis. Thorsen, *et al.*, (1997) (Surgery 122(5):882-887) demonstrated an improvement in bone density in postmenopausal women with hyperparathyroidism after parathyroidectomy.

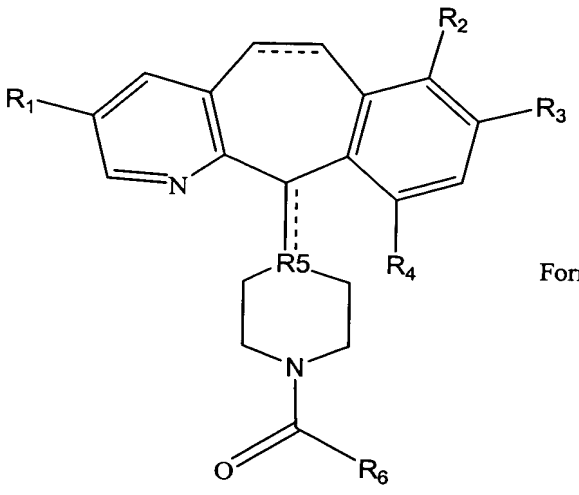
Subjects suffering from any of the above-mentioned conditions (*e.g.*, FHH, NSHPT, renal secondary hyperparathyroidism, MAH, HHM or osteoporosis) may benefit from a therapy which leads to a decrease in serum Ca^{2+} and/or serum parathyroid hormone levels. The present invention provides, *inter alia*, methods for treating disorders

of calcium homeostasis in a subject, such as those discussed above, by administering a tricyclic amide compound of the invention. Tricyclic amides, such as SCH66336, have been described previously (Liu, *et al.*, (1998) Cancer Res. 58:4974-4956; Njoroge, *et al.*, (1998) J. Med. Chem. 41:1561-1567; U.S. Patent No. 5,719,148; U.S. Patent No. 5,874,442; PCT Publication No. WO95/10516) and found to inhibit Farnesyl Protein Transferase (FPT) mediated farnesylation of the Ras protein and, thus, to be useful for treating malignancy.

The use of a farnesylation inhibitor, B-1086 (Nagasu, *et al.*, (1995) Cancer Res. 55:5310-5314), to treat malignancy-associated hypercalcemia (MAH) has been studied previously (Aklilu, *et al.*, (1997) Cancer Res. 57:4517-4522). Parathyroid Hormone Related Peptide (PTHRP) has been identified as a pathogenic factor in MAH (Moseley, *et al.*, (1987) Proc. Natl. Acad. Sci. USA 84:5048-5052; Stewart, *et al.*, (1987) Biochem. Biophys. Res. Comm. 146:672-678; Stewler, *et al.*, (1987) J. Clin. Invest. 80:1803-1807; Rabbani, *et al.*, (1986) Endocrinology 118:1200-1210; Li, *et al.*, (1994) Cancer Res. 53:2980-2986) and Aklilu *et al.* found Ras to enhance PTHRP production in rat 3T3 cells. Treatment of mice bearing Ras-3T3 tumors and suffering from hypercalcemia (MAH), with B-1086, resulted in a normalization of serum calcium and reduction of serum PTHRP levels. B-1086, however, is a peptidomimetic inhibitor which is chemically unrelated to tricyclic amides such as SCH66336. The effect of B-1086 on serum PTH levels was not investigated.

SUMMARY OF THE INVENTION

The present invention provides a method for treating or preventing disorders of calcium homeostasis (*e.g.*, hypercalcemia) in a subject comprising administering, to the subject, a compound according to the following Formula A:



Formula A

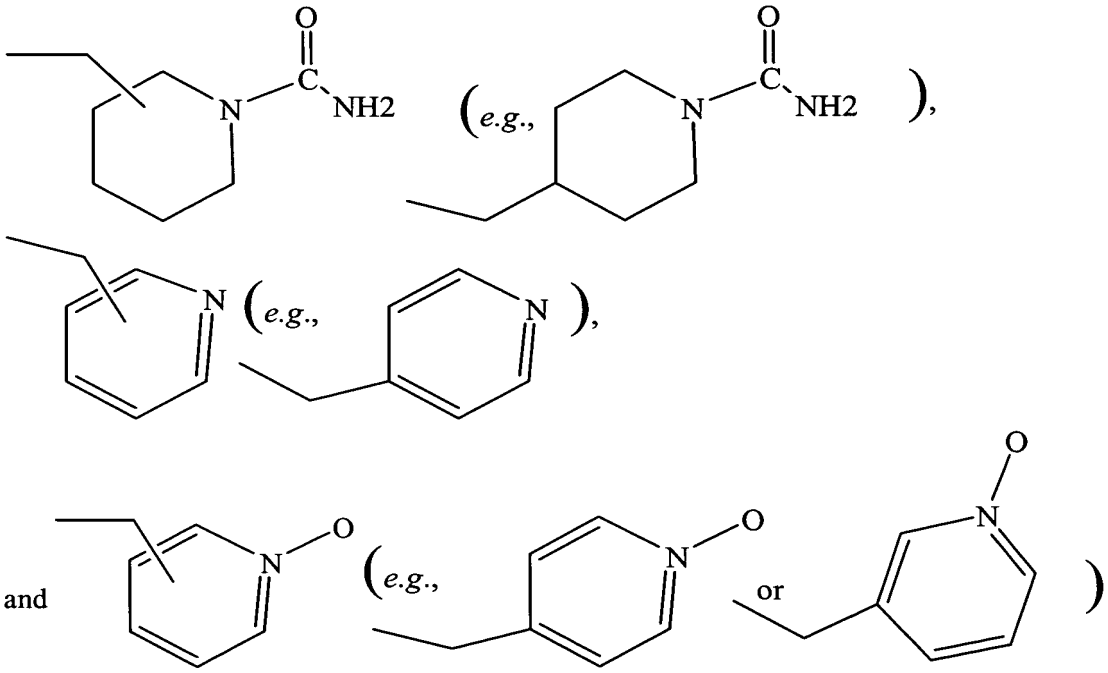
wherein R1 can be hydrogen (H) or halogen (*e.g.*, F, Cl or Br)

R2, R3 and R4 are independently selected from H and halogen (*e.g.*, F, Cl or Br) provided that at least one of R2, R3 and R4 is H;

"------" represents an optional bond;

R5 represents C when the optional bond to R5 is present and represents CH or N when the optional bond to R5 is absent;

R6 is selected from



Preferably, the compound is Formula 1 (see *infra*). Medical conditions which may be treated by the methods of the present invention include familial benign hypocalciuric hypercalcemia, neonatal severe primary hyperparathyroidism and renal secondary hyperparathyroidism.

The present invention also includes methods for agonizing the CaSR, or for decreasing the level of Ca^{2+}, PTH or PTHrP in the serum of a subject by administering a tricyclic amide compound, for example, comprising formula A, preferably comprising any of formulas 1-81, more preferably formula 1.

The tricyclic amide compounds of the present invention may be administered in association with a second substance for treating or preventing a calcium homeostatic disorder (*e.g.*, AMG073, NPS467, NPS568, gadolinium, lanthanum, neomycin, Mg^{2+},

1,25-dihydroxyvitamin D, calcitrol, paricalcitrol, doxercalciferol, zoledronic acid, calcitonin, alfacalcidol or oxacalcitriol).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides, generally, methods for treating disorders of calcium homeostasis (*e.g.*, hypercalcemia). Specifically, the present invention provides methods for treating calcium homeostatic disorders by administering a tricyclic amide of the invention (*e.g.*, Formula 1) to a subject in need of such treatment. Calcium homeostatic disorders which may be treated by administering the tricyclic amides of the present invention (*e.g.*, Formula 1) include disorders associated with excess serum Ca^{2+} and/or PTH and/or with an excess mobilization of Ca^{2+} from the bones. Such disorders include, but are by no means limited to, familial benign hypocalciuric hypercalcemia, neonatal severe primary hyperparathyroidism, renal secondary hyperparathyroidism, osteoporosis, malignancy-associated hypercalcemia (MAH) and humoral hypercalcemia of malignancy (HHM). Without being bound by a single theory, some calcium homeostatic disorders (*e.g.*, hypercalcemia) may be caused by, for example, an excess of serum PTH, or PTHrP or a lack of expression or activity of the CaSR. Again, without being bound by a single theory, the tricyclic amides of the present invention (*e.g.*, Formula 1) may treat calcium homeostasis disorders (*e.g.*, hypercalcemia) by, for example, agonizing the CaSR (*e.g.*, CaSR in the parathyroid gland or in the kidney) which may, in turn, lead to a decrease in serum PTH or PTHrP. The present invention is not limited by any particular mechanism by which a tricyclic amide of the invention (*e.g.*, Formula 1) may treat or prevent a calcium homeostatic disorder.

The term "Calcium Ion Sensing Receptor", "Calcium Sensing Receptor" or "CaSR" refers to a receptor which is commonly known in the art. A typical human CaSR amino acid sequence is set forth in SEQ ID NO: 1 as well as under Genbank Accession No. U20759, U20760 and D50855 (Garrett, *et al.*, (1995) J. Biol. Chem. 270(21): 12919-12925; Aida, *et al.*, (1995) Biochem. Biophys. Res. Comm. 214(2):524-529). A typical rat CaSR amino acid sequence is set forth in SEQ ID NO: 2 as well as under Genbank Accession No. U10354 (Riccardi, *et al.*, (1995) Proc. Natl. Acad. Sci. USA 92 (1):131-135). The term includes receptors from any species, preferably mammalian species (*e.g.*, rat, mouse, monkey, rabbit, cow) and most preferably from humans.

The term "parathyroid hormone" or "PTH" is commonly known in the art and refers to the hormone secreted by the parathyroid gland. A typical, mature, human PTH is described by Varicek, *et al.*, (1983) Proc. Natl. Acad. Sci. 80 (8), 2127-2131 and

Hendy, *et al.*, (1981) Proc. Natl. Acad. Sci. 78(12): 7365-7369). A typical, mature, rat PTH is described by Schmelzer, *et al.*, (1987) Nucleic Acids Res. 15(16): 6740. The term includes PTH from any species, preferably mammalian species (*e.g.*, dog, cat, pig, rat, mouse, monkey, rabbit, cow) and most preferably from humans.

"PTHrP" refers to hormone, parathyroid hormone related protein, secreted by tumor cells which produces effects similar to those of PTH (see, for example, Suva *et al.*, (1987) Science 237 (4817):893-896).

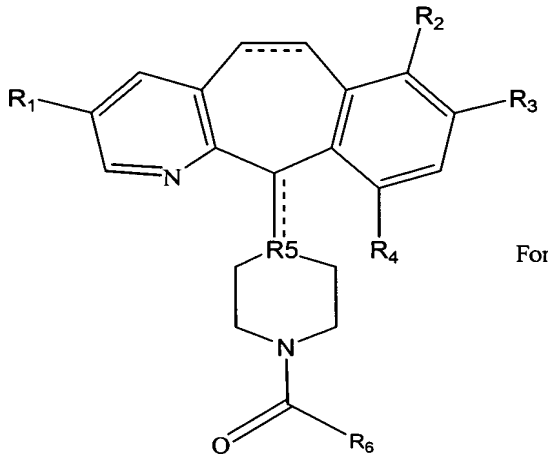
The term "subject" includes any organism, preferably a mammal (*e.g.*, dog, cat, pig, rat, mouse, monkey, rabbit, cow) and, most preferably, a human.

The term "compound" includes small molecules (*e.g.*, tricyclic amides, polymers, organic molecules, hydrocarbons, inorganic ions and salts), proteins (*e.g.*, antibodies, oligopeptides, polypeptides, hormones and enzymes), saccharides (*e.g.*, monosaccharides, oligosaccharides and polysaccharides) and nucleic acids (*e.g.*, oligonucleotides, polynucleotides, genes, plasmids, DNA and RNA).

The term " *e.g.* " means "*exempli gratia*" or "for example" and, in general, precedes one or more non-limiting examples.

Tricyclic Amides

Disorders of calcium homeostasis, in a subject, may be treated by administering a tricyclic amide compound to the subject. Preferably, the tricyclic amide compound comprises a formula set forth in U.S. Patent No. 5,719,148 or in U.S. Patent No. 5,874,442 which are herein incorporated by reference in their entireties. Preferably, the tricyclic amide compounds of the present invention include the following Formula A:



Formula A

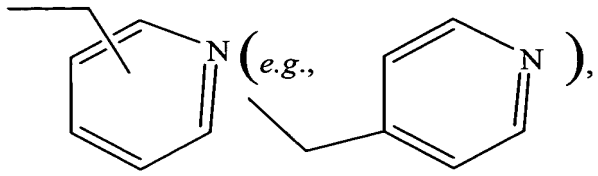
wherein R1 can be hydrogen (H) or halogen (*e.g.*, F, Cl or Br)

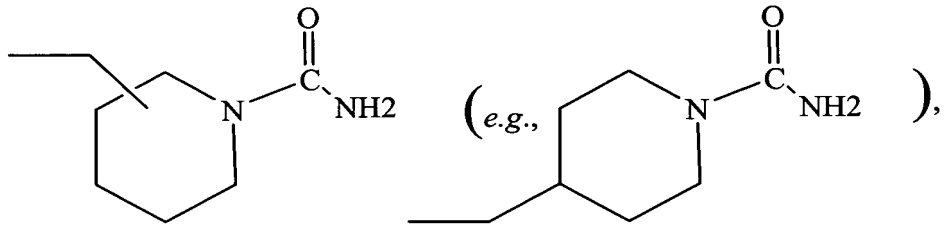
R2, R3 and R4 are independently selected from H and halogen (*e.g.*, F, Cl or Br) provided that at least one of R2, R3 and R4 is H;

"------" represents an optional bond;

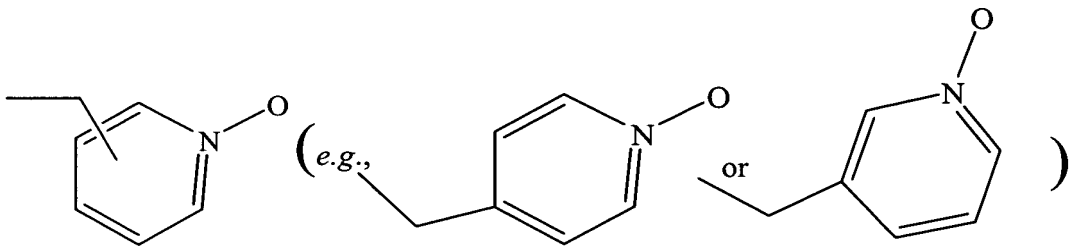
R5 represents C when the optional bond to R5 is present and represents CH or N when the optional bond to R5 is absent;

R6 is selected from





and

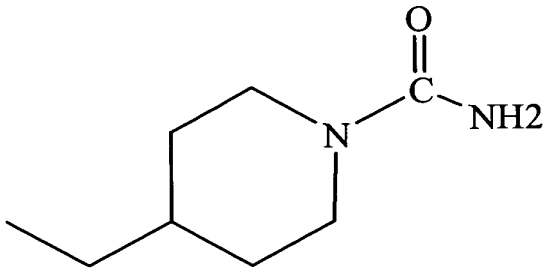


Preferably the optional bonds between C5 and C6 and on C11 (to R5), in Formula A, are absent. Preferably, R1 is halogen, more preferably Br. Representative compounds of Formula A include those wherein:

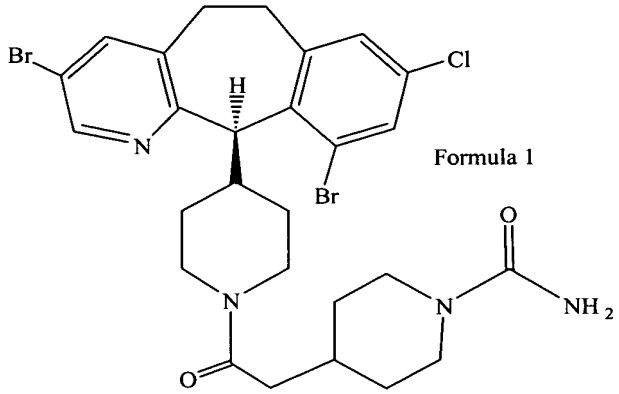
(1) R1 is halogen (preferably Br), R2 is halogen (preferably Br), R3 is halogen (preferably Cl), and R4 is H; or wherein

(2) R1 is halogen (preferably Br), R2 is H, R3 is halogen (preferably Cl) and R4 is halogen (preferably Br).

Preferably, the tricyclic amide is a compound of Formula A wherein R1 is halogen (preferably Br), R2 is H, R3 is halogen (preferably Cl) and R4 is halogen (preferably Br), the optional bond between C5 and C6 and to R5 is absent, R5 is CH and R6 is

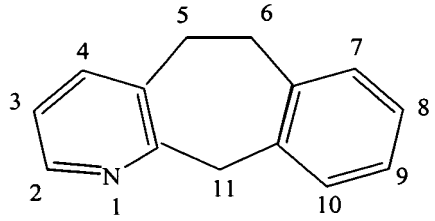


Most preferably the tricyclic amide is Formula 1:

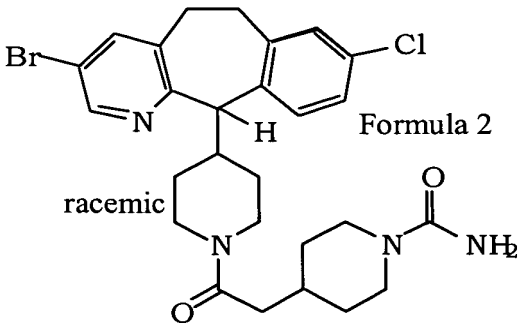


Formula 1

Those skilled in the art will appreciate that the tricyclic ring system is numbered according to the following illustration:



Other representative examples of the tricyclic amides of the present invention include but are by no means limited to:



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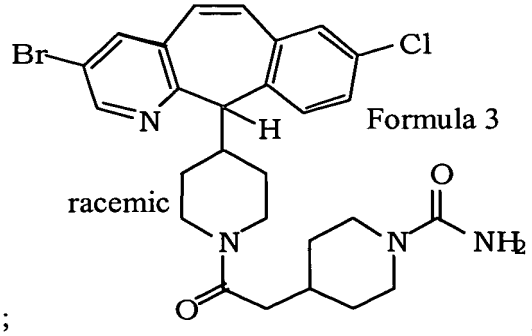

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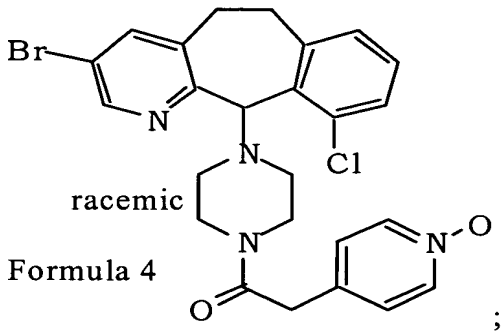

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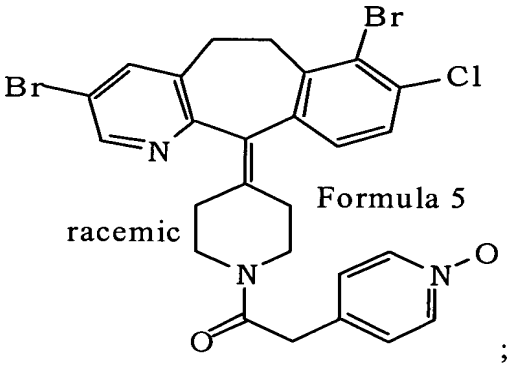

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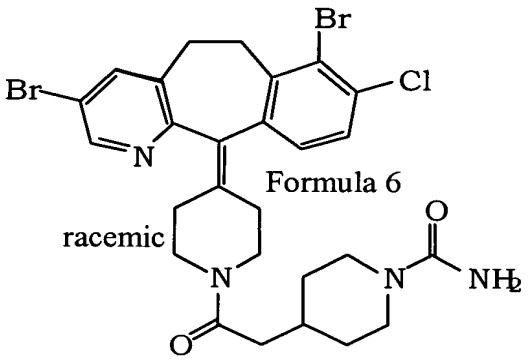

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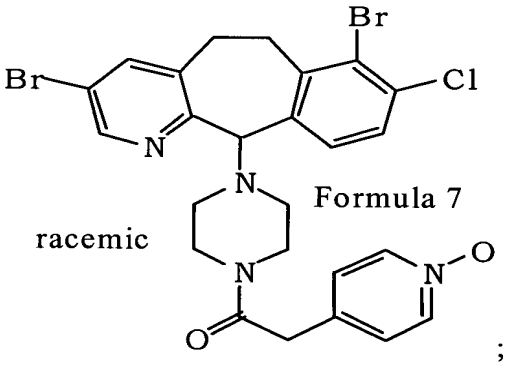

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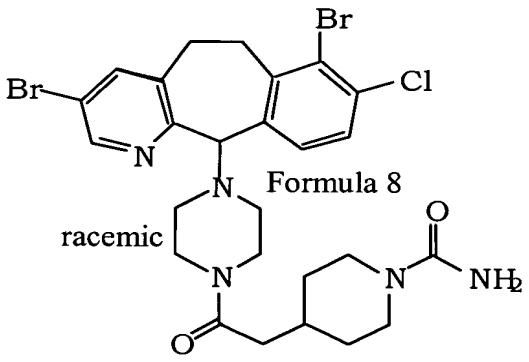

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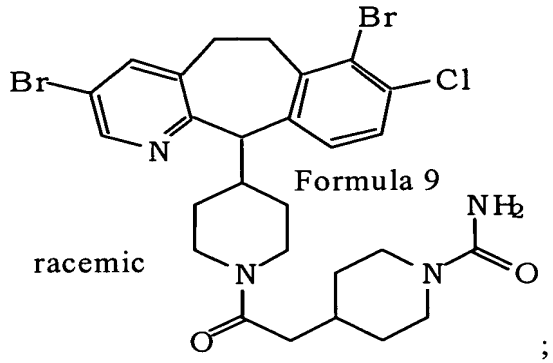

racemic

Formula 9

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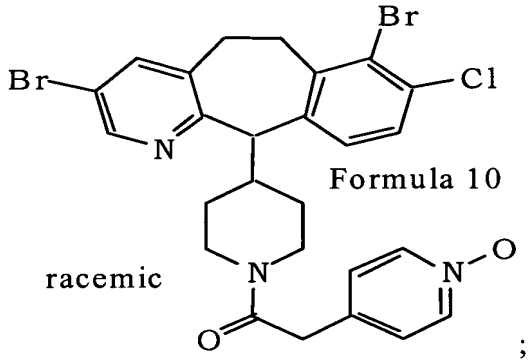

racemic

Formula 10

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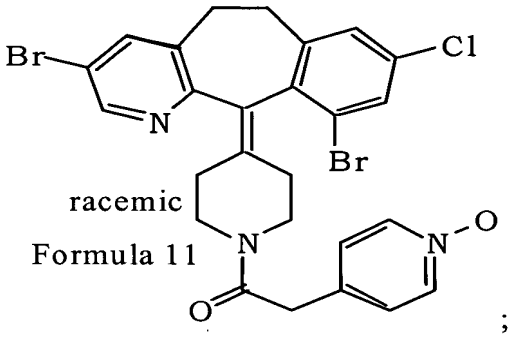

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Formula 11

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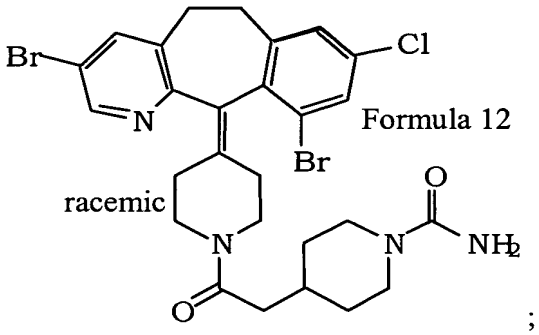

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Formula 12

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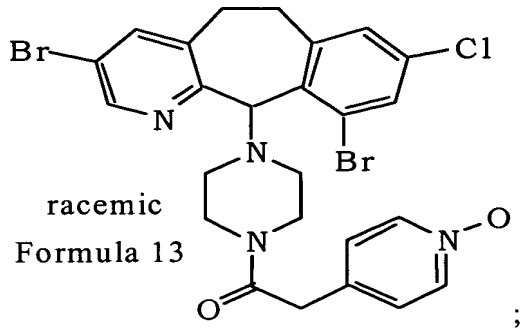

racemic
Formula 13
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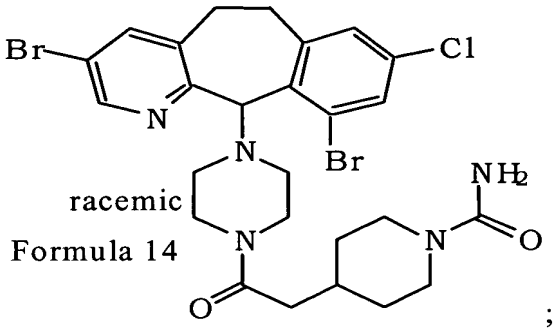

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Formula 14
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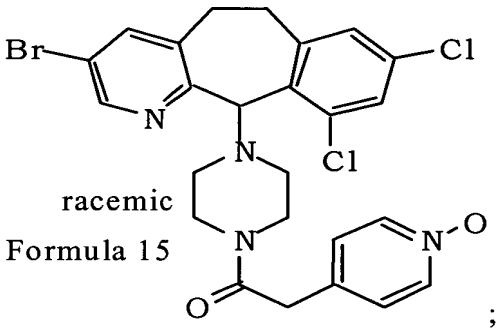

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Formula 15
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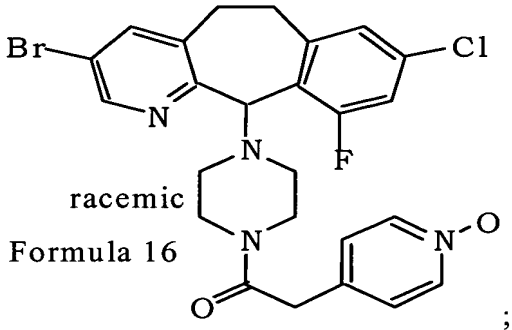

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Formula 16
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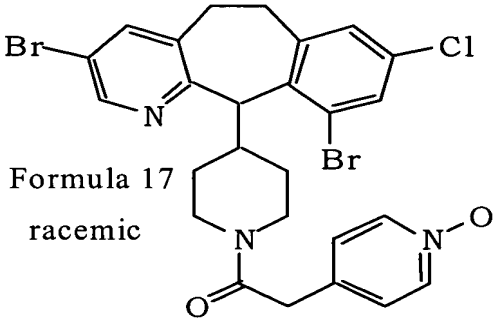

Formula 17
racemic
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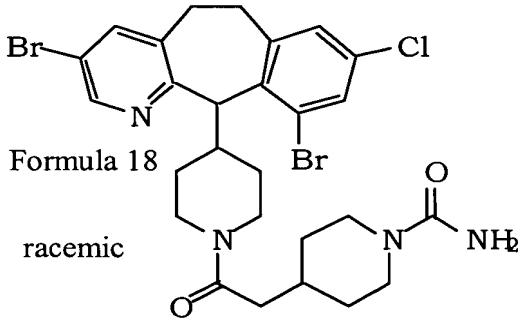

Formula 18
racemic
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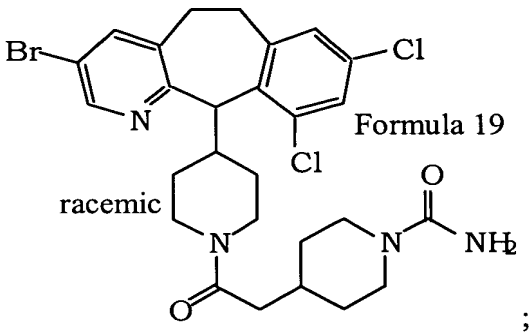
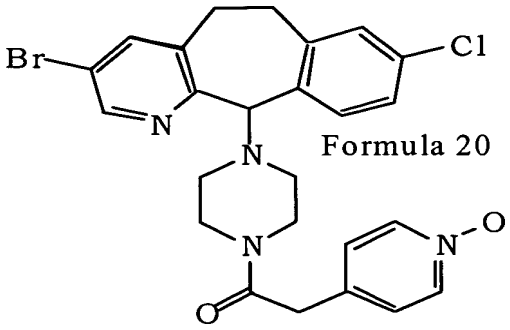


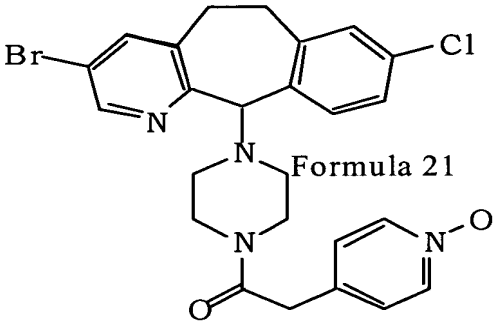
Formula 19

racemic ;



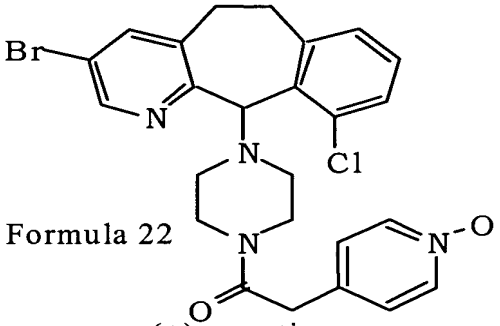
Formula 20

(+) - enantiomer ;



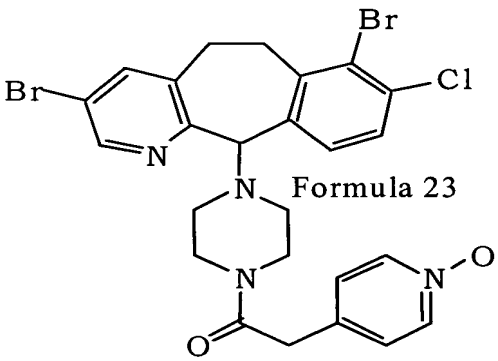
Formula 21

(-) - enantiomer ;



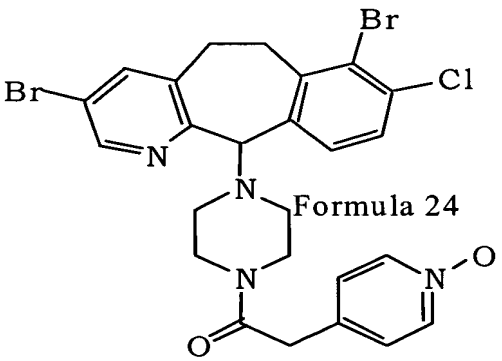
Formula 22

(+)-enantiomer ;



Formula 23

(+) - enantiomer ;



Formula 24

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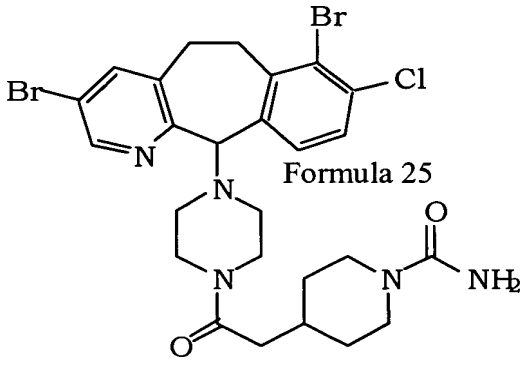

Formula 25

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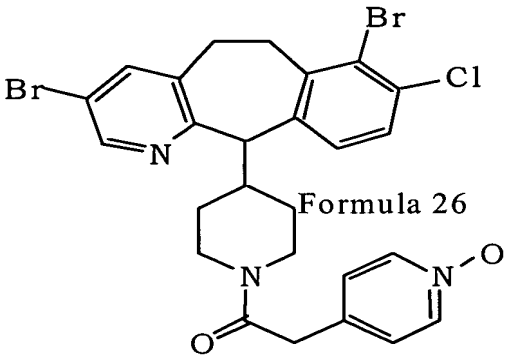
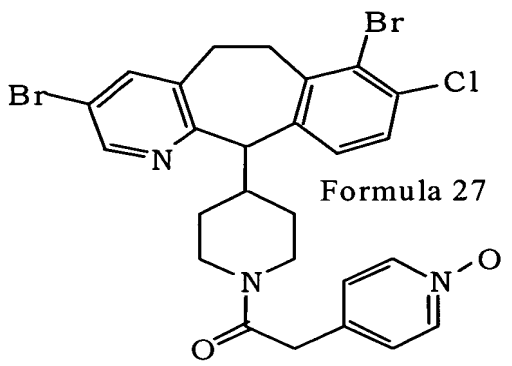


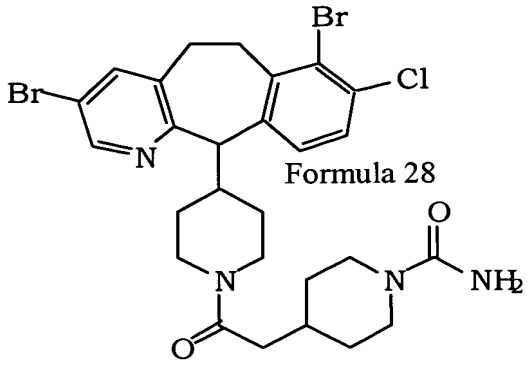
Formula 26

(+) - enantiomer ;



Formula 27

(-) - enantiomer ;



Formula 28

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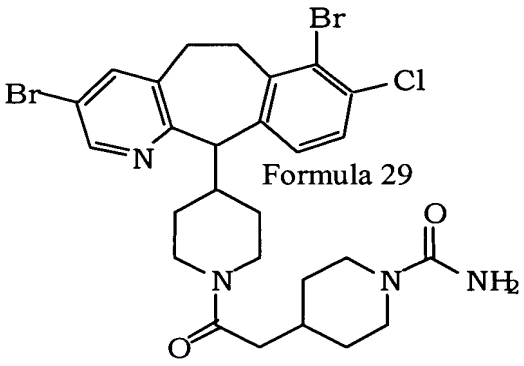
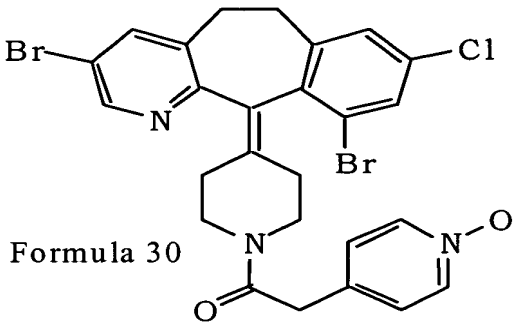


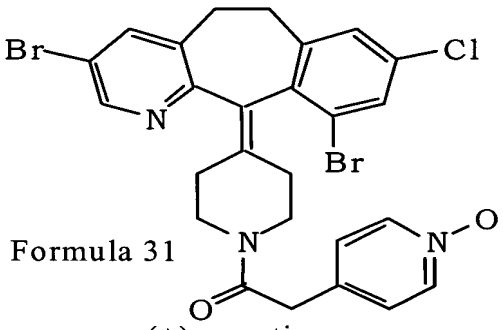
Formula 29

(-) - enantiomer ;



Formula 30

(-)-enantiomer ;



Formula 31

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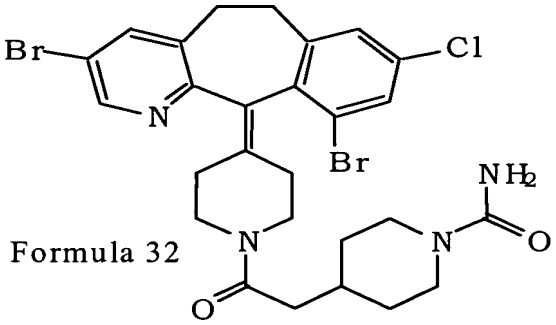
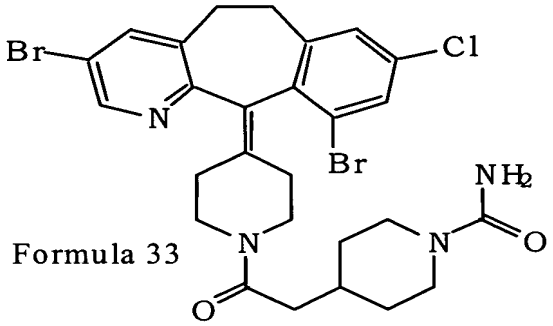


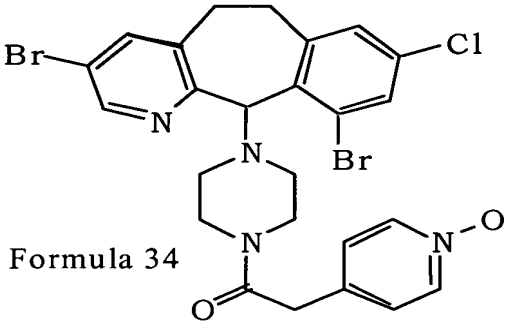
Formula 32

(-)-enantiomer ;



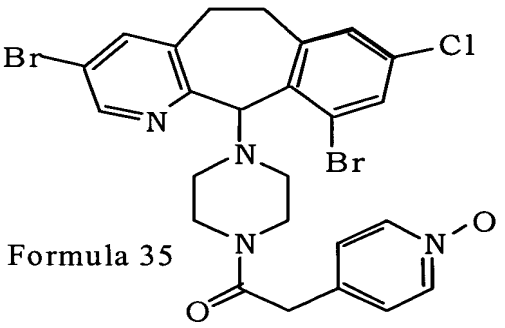
Formula 33

(+)-enantiomer ;



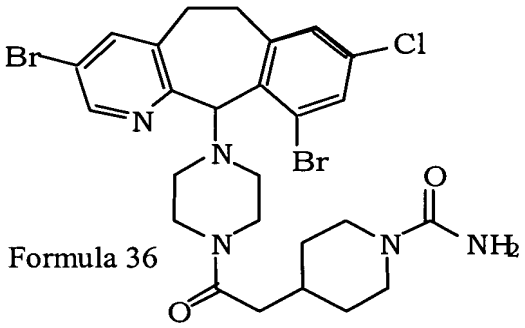
Formula 34

(+) - enantiomer ;



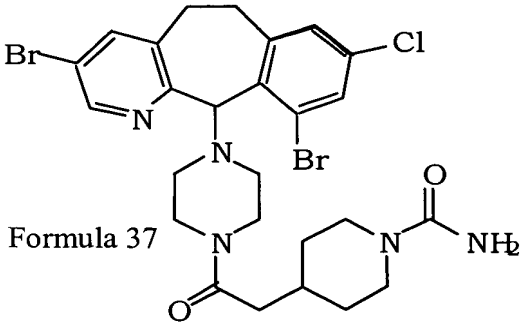
Formula 35

(-) - enantiomer ;



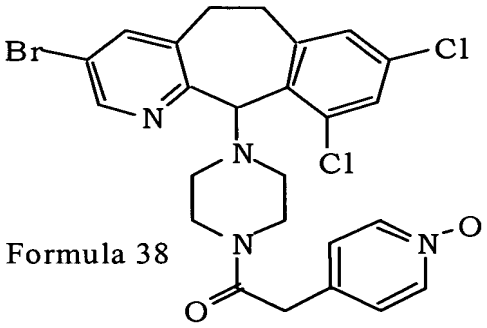
Formula 36

(+) - enantiomer ;



Formula 37

(-) - enantiomer ;



Formula 38

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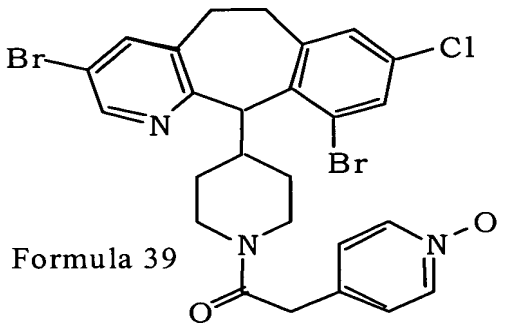

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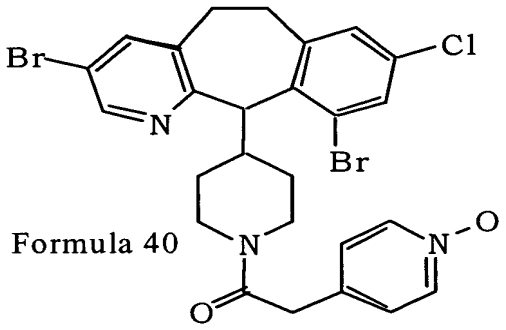
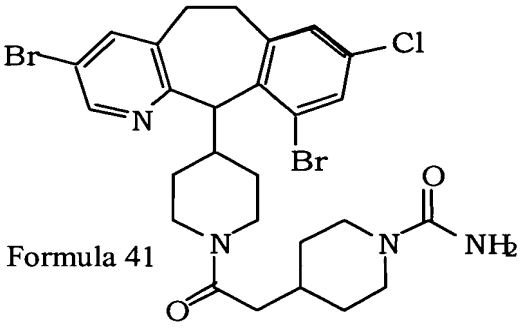
(+) - enantiomer ;



Formula 40

(-) - enantiomer ;



Formula 41

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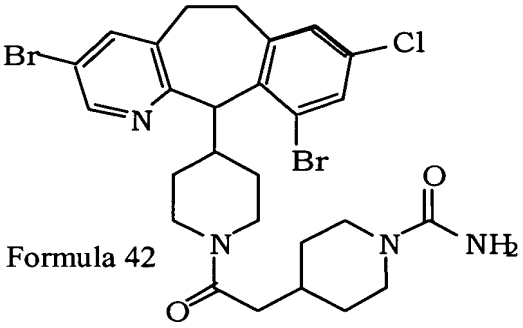
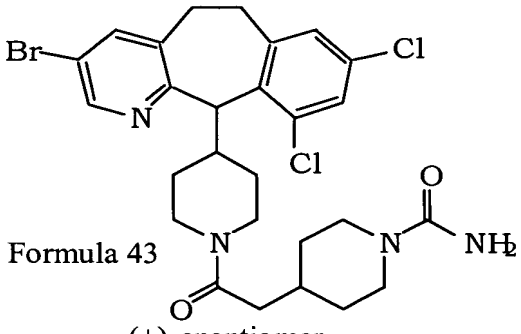


Formula 42

(-) - enantiomer ;



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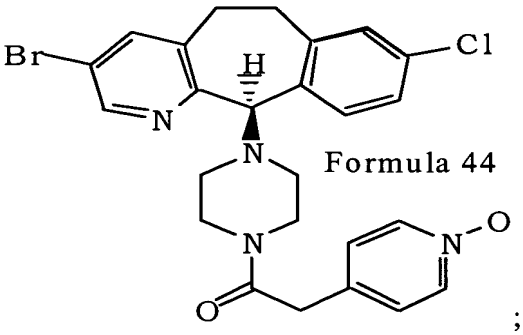

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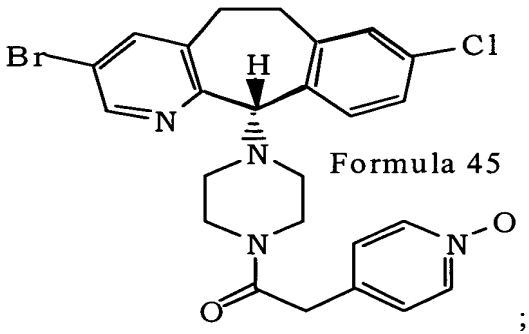

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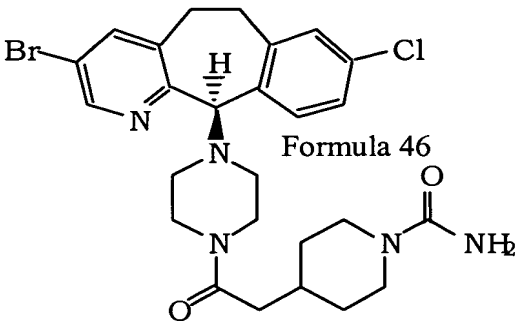
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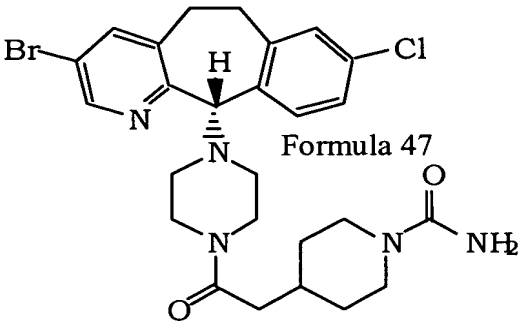
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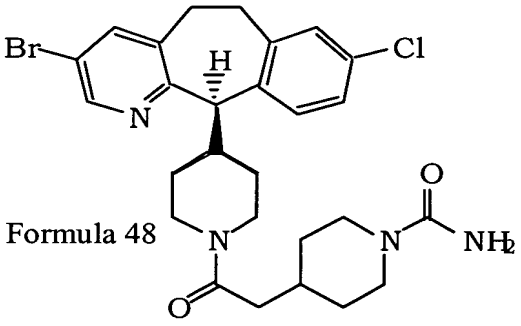
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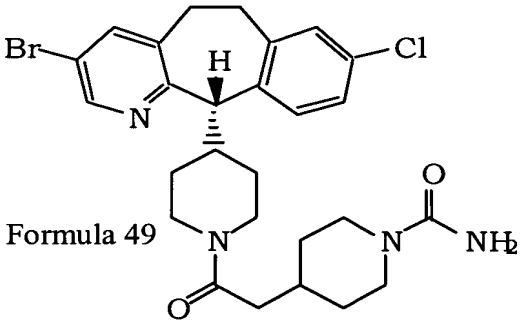
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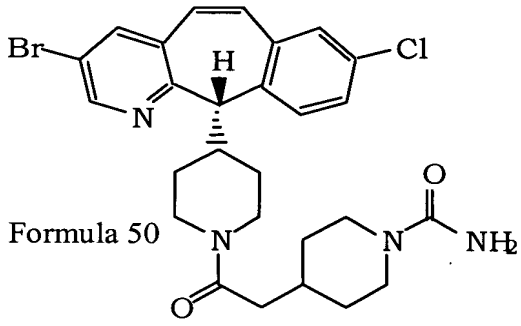
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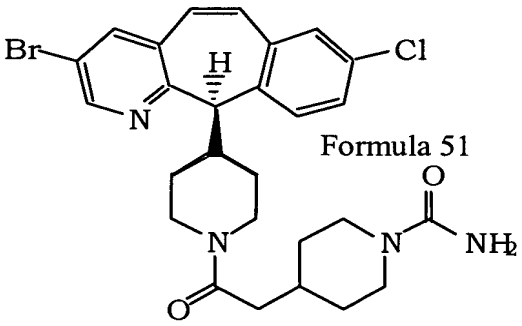
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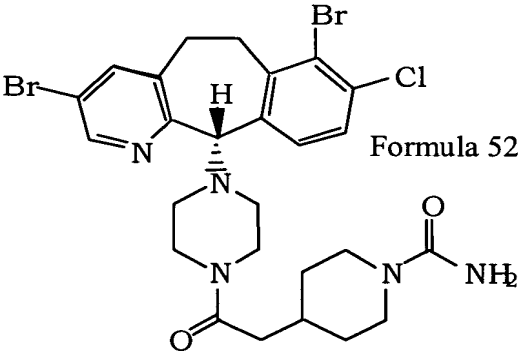
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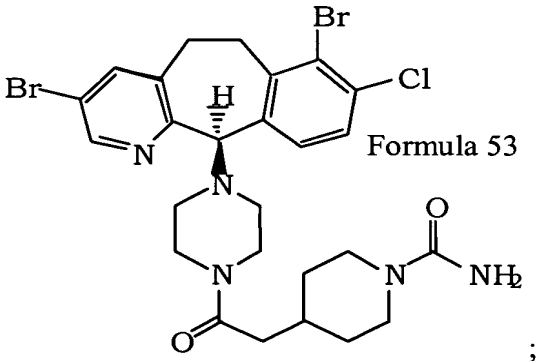
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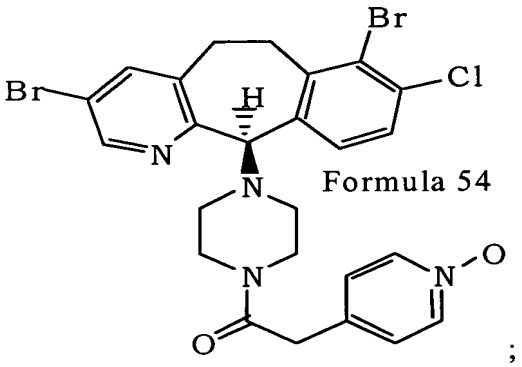
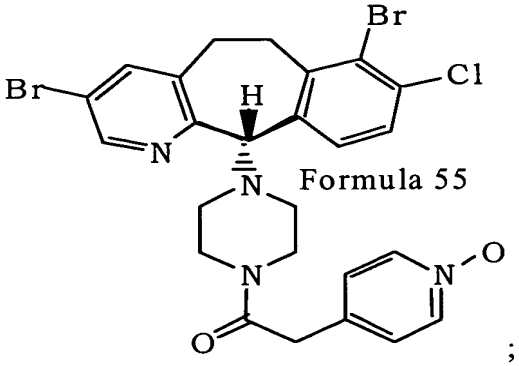


Formula 54 ;



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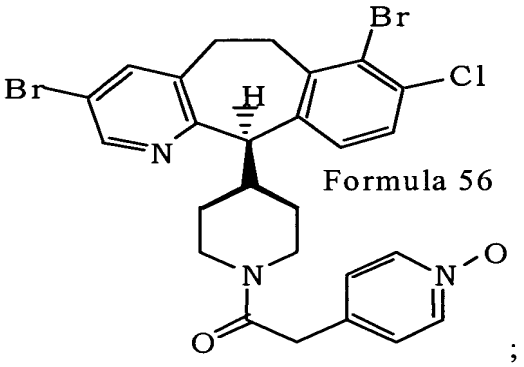

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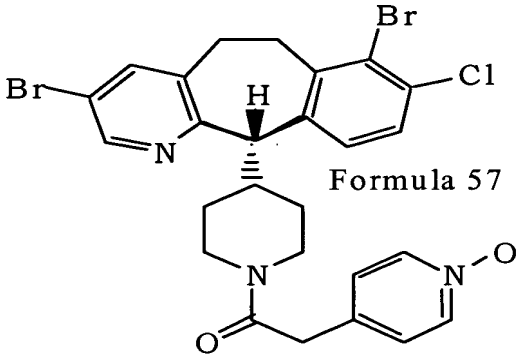

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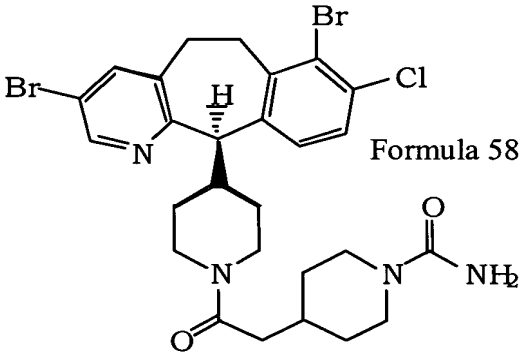

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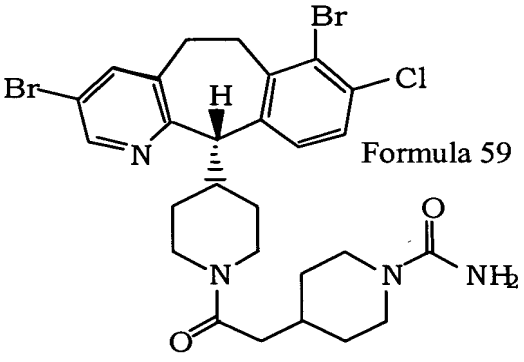

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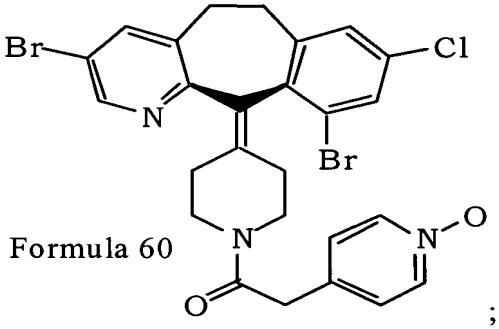

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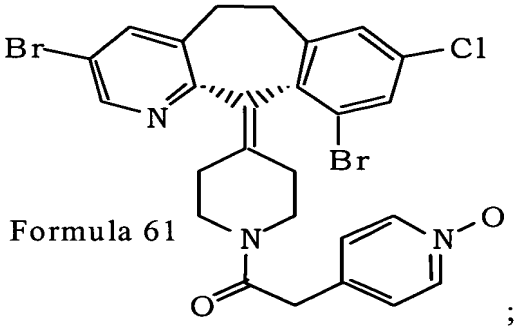

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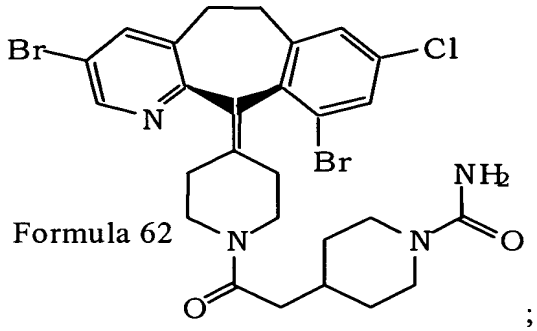

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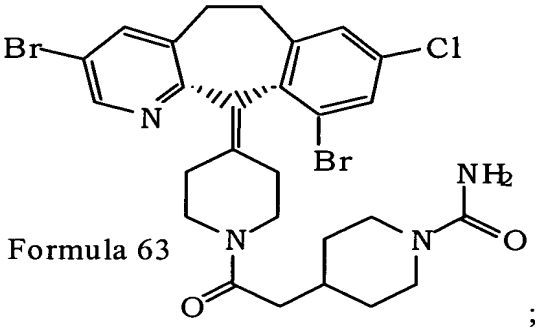

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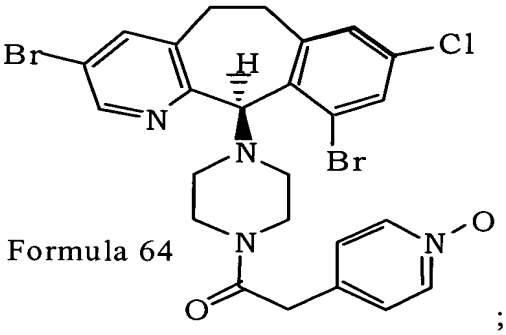

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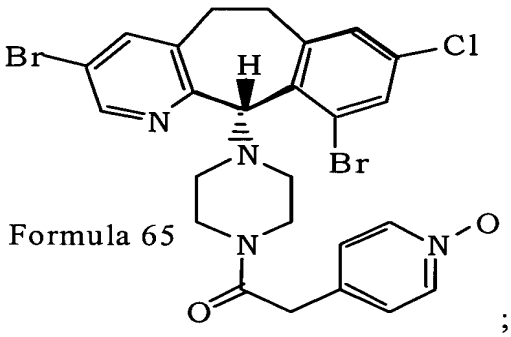

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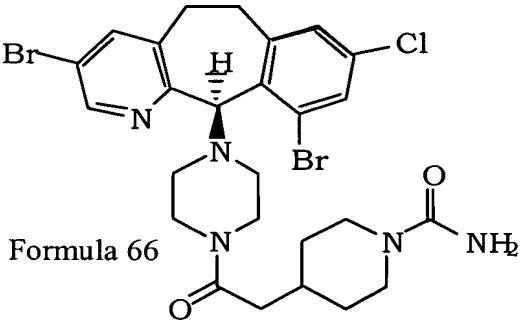

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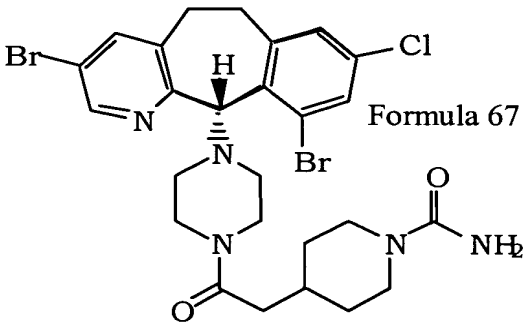

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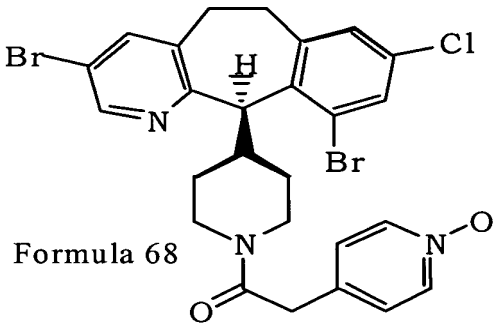

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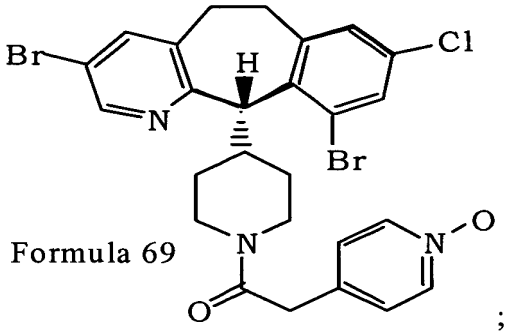

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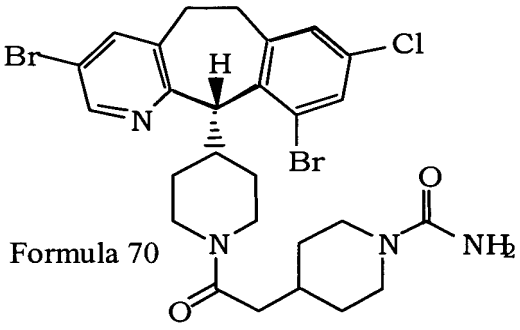

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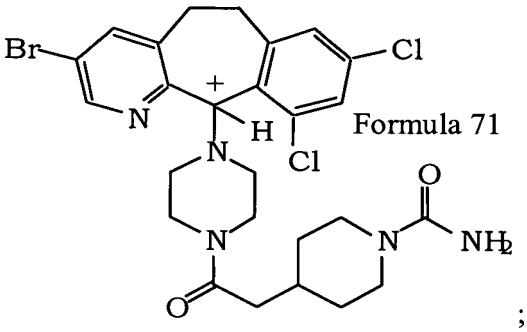
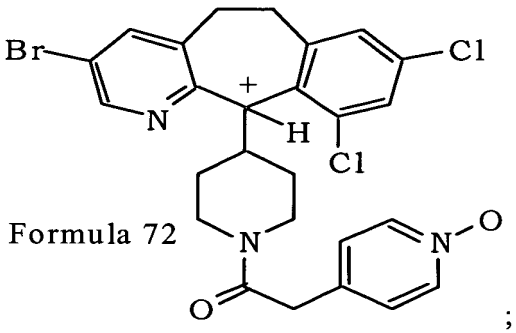


Formula 71 ;



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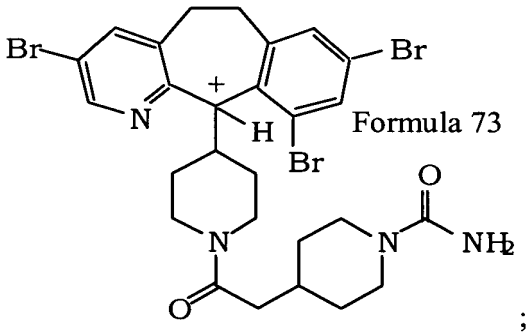

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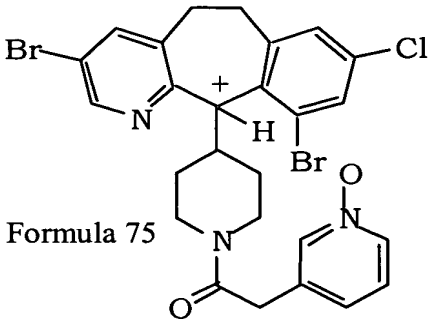
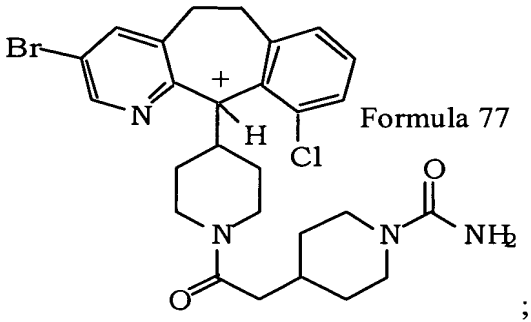
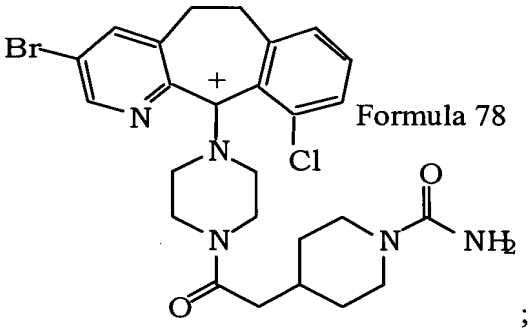


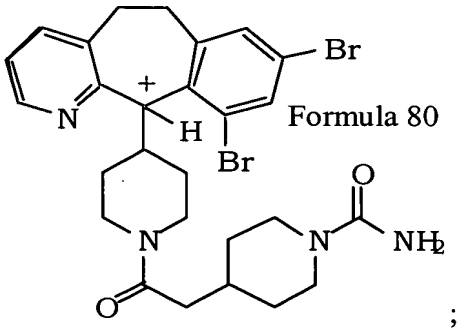
Formula 75 ;



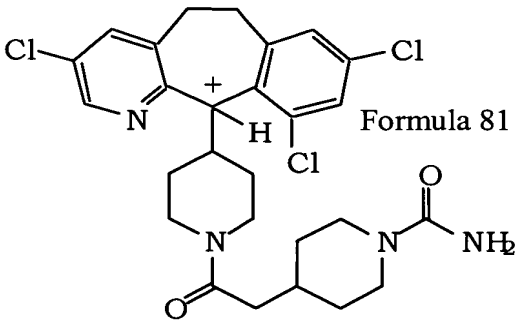
Formula 77 ;



Formula 78 ;



Formula 80 ; or



Formula 81 .

Pharmaceutical Compositions, Dosage and Administration

The present invention includes pharmaceutical compositions comprising the tricyclic amide compounds of the invention (*e.g.*, Formula 1) along with a pharmaceutically acceptable carrier.

The pharmaceutical compositions can be adapted for any mode of administration *e.g.*, for oral, parenteral, *e.g.*, subcutaneous ('SC"), intramuscular ("IM"), and intraperitoneal ("IP") or topical administration or by inhalation (*e.g.*, orally or intranasally). Preferably, tricyclic amide compounds of the invention (*e.g.*, Formula 1) are administered orally (*e.g.*, in a pill, capsule or tablet).

Such pharmaceutical compositions may be formulated by combining the tricyclic amide compounds (*e.g.*, Formula 1) or an equivalent amount of a pharmaceutically acceptable salt thereof with a suitable, inert, pharmaceutically acceptable carrier or diluent that may be either solid or liquid.

Acceptable solid form preparations include powders, tablets, dispersible granules, capsules, cachets and suppositories. The powders and tablets may be comprised of, for example, from about 1 to about 95 percent active ingredient. Suitable solid carriers are known in the art, *e.g.*, magnesium carbonate, magnesium stearate, talc, sugar or lactose. Tablets, powders, cachets and capsules can be used as solid dosage forms suitable for oral administration. Examples of pharmaceutically acceptable carriers and methods of manufacture for various compositions may be found in A. Gennaro (ed.), Remington's Pharmaceutical Sciences, 18th Edition, (1990), Mack Publishing Co.; Easton, Pennsylvania.

Liquid form preparations include solutions, suspensions and emulsions. Such preparations include, for example, water or water-propylene glycol solutions for parenteral injection. Solid form preparations may be converted into liquid preparations shortly before use for either oral or administration. Parenteral forms to be injected intravenously, intramuscularly or subcutaneously are usually in the form of sterile solutions and may contain tonicity agents (*e.g.*, salts or glucose), and buffers. Opacifiers may be included in oral solutions, suspensions and emulsions. Liquid form preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier, such as an inert compressed gas, *e.g.*, nitrogen.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, syrups, suspensions and emulsions.

Preferably, the pharmaceutical preparation is in a unit dosage form. In such form, the preparation is subdivided into suitably sized unit doses containing appropriate quantities of the active component, *e.g.*, an effective amount to achieve the desired purpose.

In general, normal total serum calcium levels range from about 8.6 mg/dl to about 10.3 mg/dl (adult human), normal serum intact PTH levels range from about 10 pg/ml to about 65 pg/ml (adult human) and normal serum ionized Ca^{2+} levels range from about 4.64 to about 5.28 mg/dl (adult human) (Tietz Fundamentals of Clinical Chemistry (5^{th} Ed); W.B. Saunders Co.; New York; 2001). Methods for measuring serum Ca^{2+} or PTH are commonly known in the art. Although it is preferable for the methods of the present invention for treating calcium homeostatic disorders to lead to normal total serum Ca^{2+} and/or serum ionized Ca^{2+} and/or PTH levels in a subject, the present invention is not so limited. The present invention includes methods for treating calcium homeostatic disorders which lead to any detectable change in total serum Ca^{2+} levels or serum ionized Ca^{2+} levels or serum PTH levels toward a normal range or which lead to any degree of alleviation of symptoms associated with the particular disorder of calcium homeostasis.

Combinations

The tricyclic amide compounds of the present invention may also be administered to a subject in association with a known, second substance which is useful for treating disorders of calcium homeostasis. Such substances include, but are not limited to, AMG073 (Goodman, *et al.*, (2002) J. Am. Soc. Nephrol. 13:1017-1024), NPS467 (Nemeth, *et al.*, (1998) Proc. Natl. Acad. Sci. 95:4040-4045), NPS568 (Nemeth, *et al.*, (1998) Proc. Natl. Acad. Sci. 95:4040-4045), gadolinium, lanthanum, neomycin, Mg^{2+}, 1,25-dihydroxyvitamin D (Delmez, *et al.*, (1989) J. Clin. Invest. 83:1349-1355), calcitrol, paricalcitrol (Martin, *et al.*, (1998) J. Am. Soc. Nephrol. 9:1427-1432), doxercalciferol (Frazao, *et al.*, (2000) Am. J. Kidney Dis. 36:562-565), zoledronic acid (*e.g.*, Zometa; Davidson, (2001) Am. J. Health Syst. Pharm. 58 Suppl. 3:S8-15), calcitonin, alfacalcidol and oxacalcitriol. Each of these substances are well known in the art.

The tricyclic amide compounds of the invention may be formulated with the second substance into a single composition or into two or more separate compositions for simultaneous consumption. Alternatively, a tricyclic amide compound of the invention

may be administered to a subject at a different time than when the second substance is administered; for example, each administration may be given non-simultaneously at several intervals over a given period of time.

EXAMPLES

Example 1: Effect of the Compound of Formula 1 on Calcium Homeostasis and PTH.

A study was undertaken to assess the potential nephrotoxicity of the compound of Formula 1 in rats. The rats were 6 week old females weighing 120.7 to 173.5 g at dosing initiation.

The study design is summarized below in Tables 1-3:

Table 1. One-Month Exploratory Nephrotoxicity Study of the Compound of Formula 1 in Female Rats: Study Design.

Group	Test/control Article	Total Daily Dose (mg/kg)	Dose Volume (ml/kg)	Dose Suspension Conc. (mg/ml)	No. Female Rats	No. Rats Bled for Plasma Analysis [1]	Duration of Dosing (days)
Control	Methyl-cellulose (0.4%; aqueous)	0	5	0	20	20[2]	3, 7, 14 or 30
Test	Formula 1	180	5	36	20	20[2]	3, 7, 14 or 30

[1] 2 hours post-dose.
[2] Blood samples were collected from five rats/group/time point on Days 2, 6, 13 and 29.

Table 2. One-Month Exploratory Nephrotoxicity Study of the Compound of Formula 1 in Female Rats: Observations and Measurements.

Investigation	Performed	Investigation	Performed
Viability and Clinical Observations	Daily beginning Week -1	Urinalysis/Urine Chemistry	Days 2, 6, 13 and 29
Body Weight	Weekly beginning	Organ Weights[1]	Days 2, 6, 13 and 29

	Week -1, and days of randomization and terminal sacrifice		
Food Consumption	Weekly beginning Week -1	Necropsy (Macroscopic Observations)	Days 2, 6, 13 and 29
Water Consumption	Days 2, 6, 13 and 29	Histopathology (Microscopic Observations)[2]	Days 2, 6, 13 and 29
Plasma analysis for Formula 1	Days 2, 6, 13 and 29 (2 hours post-dose)	Ultrastructural Pathology[3]	Days 13 and 29
Serum Chemistry	Days 2, 6, 13 and 29		

[1] Kidneys only.
[2] Kidneys and thyroid/parathyroid glands only.
[3] Parathyroid glands only.

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Table 3. One-Month Exploratory Nephrotoxicity Study of the Compound of Formula 1 in Female Rats: Assignment of Rats for Plasma Analysis, Serum Chemistry, Urinalysis, Urine Chemistry and Histopathology Evaluations.

Animal Order/Group/ Sacrifice Day	Water Consumption	Blood (ml-site of bleeding)	Plasma Analysis (ml) [1]	Serum Chemistry	Urinalysis/Urine Chemistry	Histo-pathology
1st	√	4/aorta	1.5	ADH	Std[2]	-
2nd	√	4/aorta	1.5	ADH	Std[2]	-
3rd	√	4/aorta	1.5	ADH	Std[2]	-
4th	√	4/aorta	1.5	ADH	Std[2]	-
5th	√	4/aorta	1.5	ADH	Std[2]	-
6th	√	4/aorta	-	std, PTH, vitDs, free Ca	Std[2]	K, PT
7th	√	4/aorta	-	std, PTH, vitDs, free Ca	Std[3]	K, PT
8th	√	4/aorta	-	std, PTH, vitDs, free Ca	Std[3]	K, PT
9th	√	4/aorta	-	std, PTH, vitDs, free	Std[3]	K, PT

				Ca		
10th	√	4/aorta	-	std, PTH, vitDs, free Ca	Std[3]	K, PT

[1] Blood samples were collected from five rats/group/time point on Days 2, 6, 13 and 29.
[2] Standard panel of urine chemistry except creatinine clearance (collected at room temperature).
[3] Standard panel of urine chemistry plus cAMP, GGT and NAG (collected and chilled at approximately 4°C).
ADH: Antidiuretic hormone
cAMP: Cyclic adenosine monophosphate
free Ca: Free calcium
GGT: Gamma-glutamyl transpeptidase
K: Kidneys
NAG: N-acetyl glucosaminidase
PT: Parathyroid glands
PTH: Parathyroid hormone
vitDs: 1, 25-dihydroxy vitamin D and 25-hydroxy vitamin D

Std.: Standard Panel of Serum Chemistry: Blood was collected at necropsy from the abdominal aorta. For the ten rats sacrificed at each timepoint, serum from five rats were analyzed for the standard chemistry profile and antidiuretic hormone (ADH). Serum from the remaining five rats were analyzed for parathyroid hormone, 1, 25-dihydroxy vitamin D, 25-hydroxy vitamin D and free calcium. Blood (2-4 ml/rat) was collected into a 4 ml draw red/gray top (no anticoagulant) serum separator tube and the following parameters were measured: hemolysis; lipemia; icterus; glucose; urea nitrogen; creatinine; alkaline phosphatase; total protein; albumin; globulin (calculated); albumin/globulin ratio (calculated); sodium; potassium; chloride; calcium (total); free calcium; phosphorus; parathyroid hormone; vitamin D; antidiuretic hormone (ADH); 1, 25-dihydroxy vitamin D; 25-hydroxy vitamin D.

Std.: Standard Panel of Urine Chemistry: Urine was collected and chilled at approximately 4°C from all rats by cage run-off during 24 hours preceding each scheduled necropsy. For freshly voided samples, at least 2 ml of urine were collected from each rat. For 24-hour samples, urine was collected from each rat for the remaining 24 hours after voided sample collection. All urine samples were collected in 50 ml centrifuge tubes, chilled and stored at approximately 4°C before analysis. Unused voided samples were combined with the respective 24-hour samples for urinalysis. The following parameters were measured in freshly voided samples: color; clarity; pH; protein; glucose; ketones; bilirubin; blood; urobilinogen; microscopic analysis. For 24-hour samples: sodium; potassium; chloride; calcium (total); phosphorus; creatinine clearance; volume; osmolality.

Data collected during the study are summarized, below, in Tables 4-7.

Table 4. Mean calcium and phosphorus excretion (g/kg/24hours).

	Day 2	Day 2	Day 6	Day 6	Day 13	Day 13	Day 29	Day 29
Formula 1	Ca	P	Ca	P	Ca	P	Ca	P
0 mg/kg	7.30	57.93	5.55	51.60	9.56	37.8	10.88	39.43
180 mg/kg	5.65	55.86	32.99	13.54	20.69	12.11	19.24	7.30

Table 5. Mean serum PTH (ng/ml).

Formula 1	Day2	Day 6	Day 13	Day 29
0 mg/kg	94	54	89	90
180 mg/kg	60	51	68	44

Table 6. Mean serum ADH (pg/ml).

Formula 1	Day 2	Day 6	Day 13	Day 29
0 mg/kg	193	97	115	619
180 mg/kg	350	513	547	616[1]

[1]At this timepoint, water consumption had increased, which would result in less stimulus for ADH production.

Table 7. Mean urine volume (ml/kg/24 hours) and osmolality (mOsm/kg).

	Day 2	Day 2	Day 6	Day 6	Day 13	Day 13	Day 29	Day 29
Formula 1	Vol.	Osmo	Vol.	Osmo	Vol.	Osmo	Vol.	Osmo
0 mg/kg	75	1541	71	1573	100	1176	84	1073
180 mg/kg	124	646	195	646	177	655	166	606

Within 6 days of dosing at 180 mg/kg, calcium excretion had increased in the urine and phosphorus excretion was decreased. Furthermore, a decrease in serum PTH was observed in rats dosed with the compound of Formula 1 and, within 28 days, 5 out of 5 rats evaluated had lower PTH serum concentrations than did the control rats. Decreases in urine osmolality, increases in urine volume and increases in antidiuretic hormone (ADH) concentrations suggest the development of nephrogenic diabetes insipidus (failure of kidneys to concentrate urine in response to ADH).

These data also strongly suggest that structurally related tricyclic amide compounds (*e.g.*, formulas 2-81or those disclosed in U.S. Patent No. 5,719,148 or in U.S. Patent No. 5,874,442) would exhibit *in vivo* properties which are similar to those described above for the compound of Formula 1. Specifically, the structurally related tricyclic amide compounds should also be useful for treating calcium homeostatic disorders such as hypercalcemia.

Without being bound by a single theory, agonism of the CaSR in the parathyroid gland of a subject (*e.g.*, by administration of Formula 1) may lead to lower serum parathyroid hormone levels which in turn may lead to lower Ca^{2+} serum levels. The parathyroid cells may decrease PTH secretion by fusing secretory granules containing PTH with lysosomes; this would lead to PTH degradation. Again, without being bound by a single theory, at the kidney level, the Calcium Ion Sensing Receptor may help regulate calcium ion reabsorption. Calcium regulation at the kidney may be independent of parathyroid hormone (Brown, *et al.*, (1995) N. Engl. J. Med. 333(4):234-240). Under this theory, when the Calcium Ion Sensing Receptor is activated by increased calcium ions in the plasma, there is a diminished uptake of calcium from the filtrate, resulting in increased calciuria.

Example 2: Micrographic Analysis of Rat Parathyroid Glands.

Microscopic evaluation of the parathyroid glands of rats dosed with 180 mg/kg Formula 1 revealed cytoplasmic vacuolation.

Electron photomicrographs of the right parathyroid gland from two control rats sacrificed on Day 13 (Nos. 26F and 29F), two control rats sacrificed on Day 29 (Nos. 39F and 40F) and two Formula 1-dosed rats sacrificed on Day 29 (Nos. 76F and 77F) were examined for ultrastructural changes. The majority of parathyroid chief cells from the two Formula 1-dosed rats sacrificed on Day 29 had less conspicuous cell borders with fewer, less distinctive cytoplasmic projections. This suggests that the gland from which the cells were obtained was less active in PTH production/secretion. Consistent with the light microscopic appearance, the cytoplasm contained numerous, generally membrane-bound vesicles, approximately 0.2-3 μm in diameter, that were generally electron lucent with occasional irregular, variably electron-dense material. The number of myeloid bodies and fat globules was minimally increased. A single rounded parathyroid epithelial cell in animal No. 77F appeared apoptotic with condensed chromatin and intact mitochondria and contained numerous vesicles similar to those previously described. The apoptotic cell is consistent with the single cell necrosis observed at the light microscopic

level. This too suggests that the gland from which the cells were obtained was less active in PTH production/secretion.

Considering the increased number of vesicles and myeloid bodies in the Formula 1-dosed rats, some vesicles may have fused with lysosomes (crinophagy) which would lead to a decrease in PTH secretion. There was no evidence of exhaustion vacuoles. Additionally, there were no changes indicating chronic stimulation such as increased rough endoplasmic reticulum and prominent Golgi apparatus.

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Patents, patent applications, publications, Genbank submission accession numbers, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.